

REVIEWED

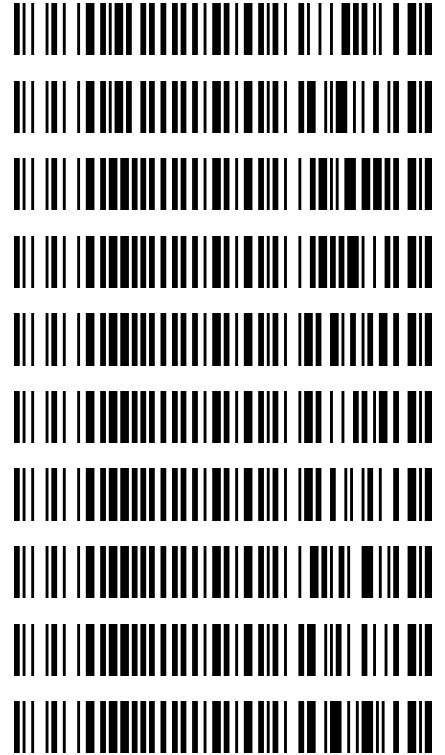
By Sarah Collins at 8:15 am, Sep 13, 2021



8/23/2021

Worklist: 5193

<u>LAB_CASE</u>	<u>ITEM</u>	<u>ITEM_TYPE</u>	<u>DESCRIPTION</u>
M2021-3441	1	BCK	AM 27 Blood THC Quant by LC-QQQ
M2021-3588	4	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-2642	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-2644	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-2646	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-2647	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-2648	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-2673	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-2718	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-2720	1	BCK	AM 27 Blood THC Quant by LC-QQQ



AK

8/31/2021

Worklist: 5205

<u>LAB_CASE</u>	<u>ITEM</u>	<u>ITEM_TYPE</u>	<u>DESCRIPTION</u>
P2021-2522	3	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-2669	1	BCK	AM 27 Blood THC Quant by LC-QQQ



AM# 27: Quantitation of THC and Metabolites in Blood and Urine by LC-MS/MS

Extraction Date: 09/09/2021

Analyst: Amber Gerheart

Plate lot#: 210609

Plate Retest Date: 12/09/2021

Mobile phase A: 0.1% Formic Acid in LCMS Water

Mobile phase B: 0.1% Formic acid in Acetonitrile

Blank Blood Lot: Lampire 20L20724

Column: UCT Selectra DA 100 x 2.1mm 3um

LCMS-QQQ ID: 069901

Blank Urine Lot: N/A

Pre-Analytic:

- 1. Check levels of mobile phases and needle wash refill as needed. Ensure waste is not full.
- 2. Ensure correct column is installed and begin mobile phase flow allow to equilibrate ~ 30 minutes.

Analytic:

- 1. Remove standards, plate, controls, and samples from cold storage. Allow to reach room temperature.
- 2. Urine hydrolysis: add 1.5mL urine to blank plate, add 250µl 1N KOH. Shake and incubate at 40 degrees for 15 minutes. Using a calibrated pipette, add **1000µl blood and urine (if applicable) (calibrated pipette)** into the appropriate wells of analytical (standards) plate. **Pipette ID: 42**
- 3. Place on shaking incubator at ambient temp., 900rpm for 15 minutes.
- 4. Pipette **500µL 0.1% formic acid in water blood sample, 500 µL saturated phosphate buffer in urine** in wells of analytical plate.
- 5. Place on shaking incubator at ambient temp., 900rpm for 15 minutes.
- 6. Transfer **700-800µL of blood+acid or urine+acid** mixture to corresponding wells of SLE+ plate.
Amount transferred: 800 µL
- 7. Apply positive pressure for approx. 10-15 seconds (or until no liquid remains on top of sorbent).
(Load at 85-100 PSI- Selector to the right)
- 8. Wait 5 minutes.
- 9. Add **2.25mL MTBE. (Add in 3 increments of 750uL)**
- 10. Wait 5 minutes.
- 11. Apply positive pressure for approx. 15 seconds. **(10-15 PSI- Selector to the left).**
- 12. Add **2.25mL Hexane. (Add in 3 increments of 750uL)**
- 13. Wait 5 minutes.
- 14. Apply positive pressure for approx. 15 seconds. **(10-15 PSI- Selector to the left).**
- 15. Remove plate containing eluate. Place on SPE Dry and evaporate to dryness at approx. 35°C.
- 16. Reconstitute in **100µL 100% MeOH** and heat seal plate with foil. Place in autosampler and run worklist.

Post-Analytic

- 1. Create batch and process data.
- 2. Make any necessary integration changes, Curve weighting of Linear 1/x with r^2 values ≥ 0.98 for each analyte
- 3. RT +/- 3% or 0.100 min, whichever is greater, +/- 20% Accuracy for greater than (+/- 30% for 10ng/ml or less).
Ion ratios must be within +/- 20% of the averaged calibrators
- 4. Case samples with calculated concentrations for THC at 1ng/mL or greater and OH-THC at 3ng/mL or greater may be reported quantitatively (blood only). Calculated concentrations for carboxy-THC of 5ng/mL may be reported qualitatively. Samples with a THC or OH-THC response over 50 ng/mL will be reported out as greater than 50 ng/mL.
- 5. Did all QCs pass for each analyte? (if not, describe in comments section)
- 6. Enter QCs into control charting.
- 7. Central File Packet to include: LIMS Worklist, Method Checklist, Calibration and Control Reports

COMMENTS: Checklist used for worklist 5193 and 5205

THC-OH not evaluated due to a possible interfering compound.

	1	2	3	4	5	6
A	IS + Cal. 1	Blood Negative	P2021-2648-1	IS + Sample	IS + Sample	IS + QC_1
B	IS + Cal. 2	M2021-3441-1	P2021-2669-1	IS + Sample	IS + Sample	IS + Cal. 7
C	IS + Cal. 3	M2021-3588-4	P2021-2673-1	IS + Sample	IS + Sample	IS + Cal. 6
D	IS + Cal. 4	P2021-2522-3	P2021-2718-1	IS + Sample	IS + Sample	IS + Cal. 5
E	IS + Cal. 5	P2021-2642-1	P2021-2720-1	IS + Sample	IS + Sample	IS + Cal. 4
F	IS + Cal. 6	P2021-2644-1	IS + Sample	IS + Sample	IS + Sample	IS + Cal. 3
G	IS + Cal. 7	P2021-2646-1	IS + Sample	IS + Sample	IS + Sample	IS + Cal. 2
H	IS + QC_1	P2021-2647-1	IS + Sample	IS + Sample	IS + Sample	IS + Cal. 1

All wells to contain 100 µl of residual DMSO

AG

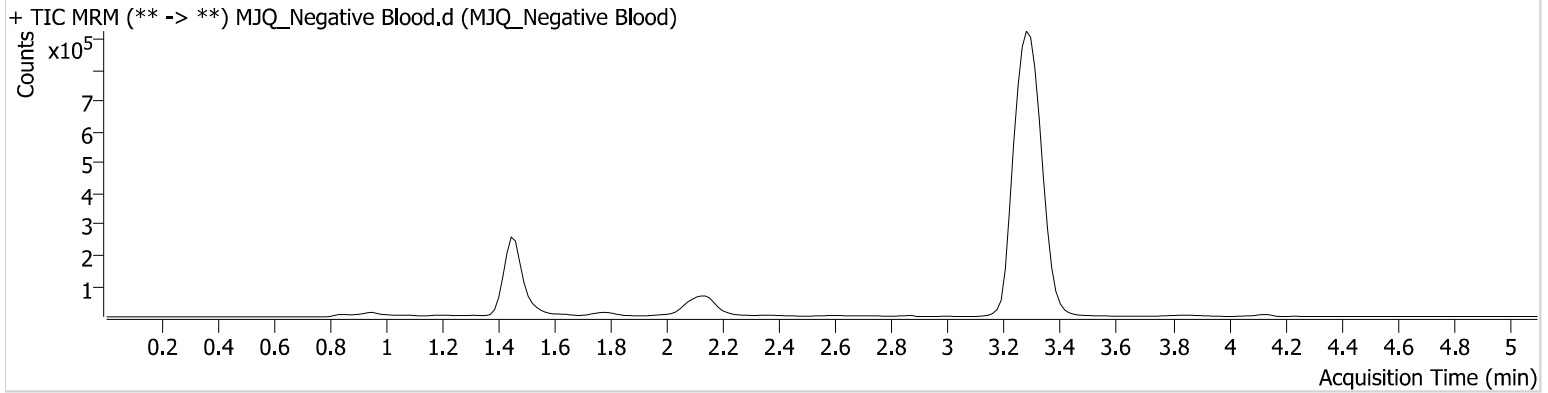


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\090921 AM 27 AG\QuantResults\AM 27.batch.bin
Calibration Last Update 9/10/2021 2:52:11 PM

Instrument	Falco (069901)	Data File	MJQ_Negative Blood.d
Type	Sample	Sample	MJQ_Negative Blood
Acq. Method	AM 27 THCQ.m	Operator	Amber Gerheart
Sample Position	P1-A2	Comment	
Injection Volume	10		
Acq. Date-Time	9/9/2021 1:49:37 PM		
Sample Info.			

Sample Chromatogram



AG

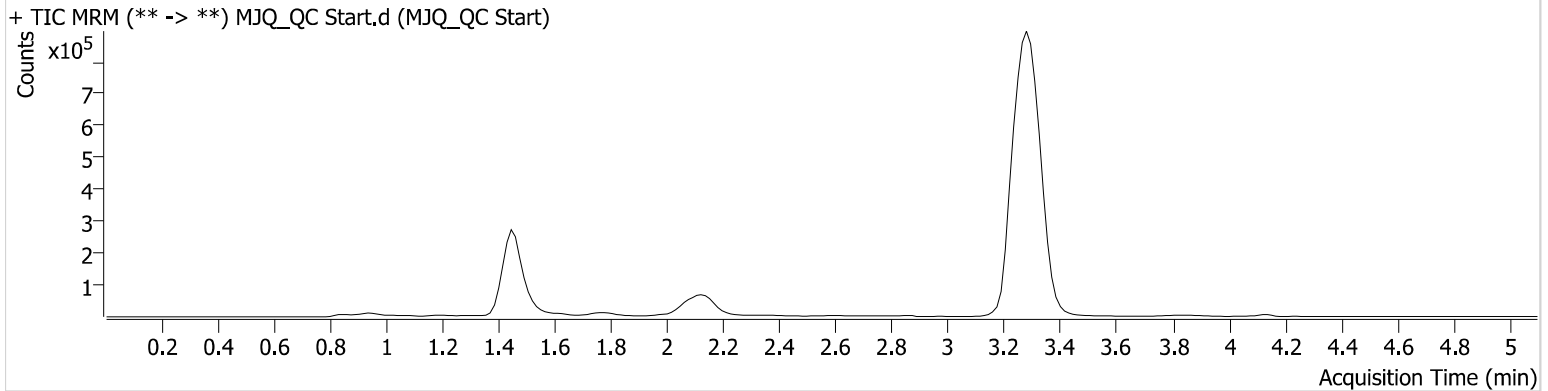


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\090921 AM 27 AG\QuantResults\AM 27.batch.bin
Calibration Last Update 9/10/2021 2:52:11 PM

Instrument	Falco (069901)	Data File	MJQ_QC Start.d
Type	Sample	Sample	MJQ_QC Start
Acq. Method	AM 27 THCQ.m	Operator	Amber Gerheart
Sample Position	P1-H1	Comment	
Injection Volume	10		
Acq. Date-Time	9/9/2021 1:34:25 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.468	128450	∞	6.8 Low	41.90	904913	4.5699 ng/ml
THC-COOH	1.474	75798	∞	63.8	1045.65	198946	14.8005 ng/ml
THC	3.300	262784	1555.45	27.5	∞	5900018	4.7973 ng/ml

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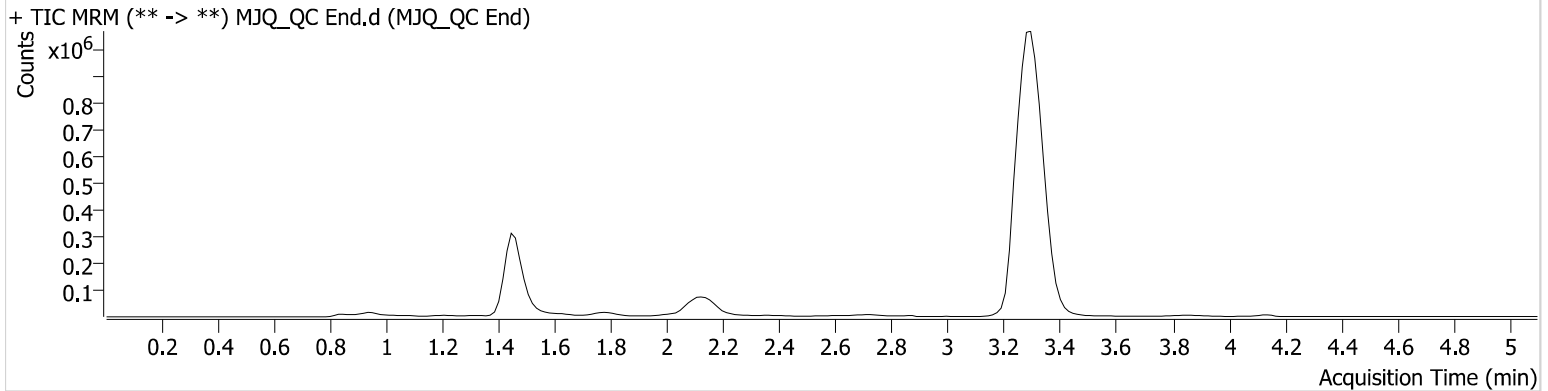


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\090921 AM 27 AG\QuantResults\AM 27.batch.bin
Calibration Last Update 9/10/2021 2:52:11 PM

Instrument	Falco (069901)	Data File	MJQ_QC End.d
Type	Sample	Sample	MJQ_QC End
Acq. Method	AM 27 THCQ.m	Operator	Amber Gerheart
Sample Position	P1-H1	Comment	
Injection Volume	10		
Acq. Date-Time	9/9/2021 5:07:25 PM		

Sample Chromatogram



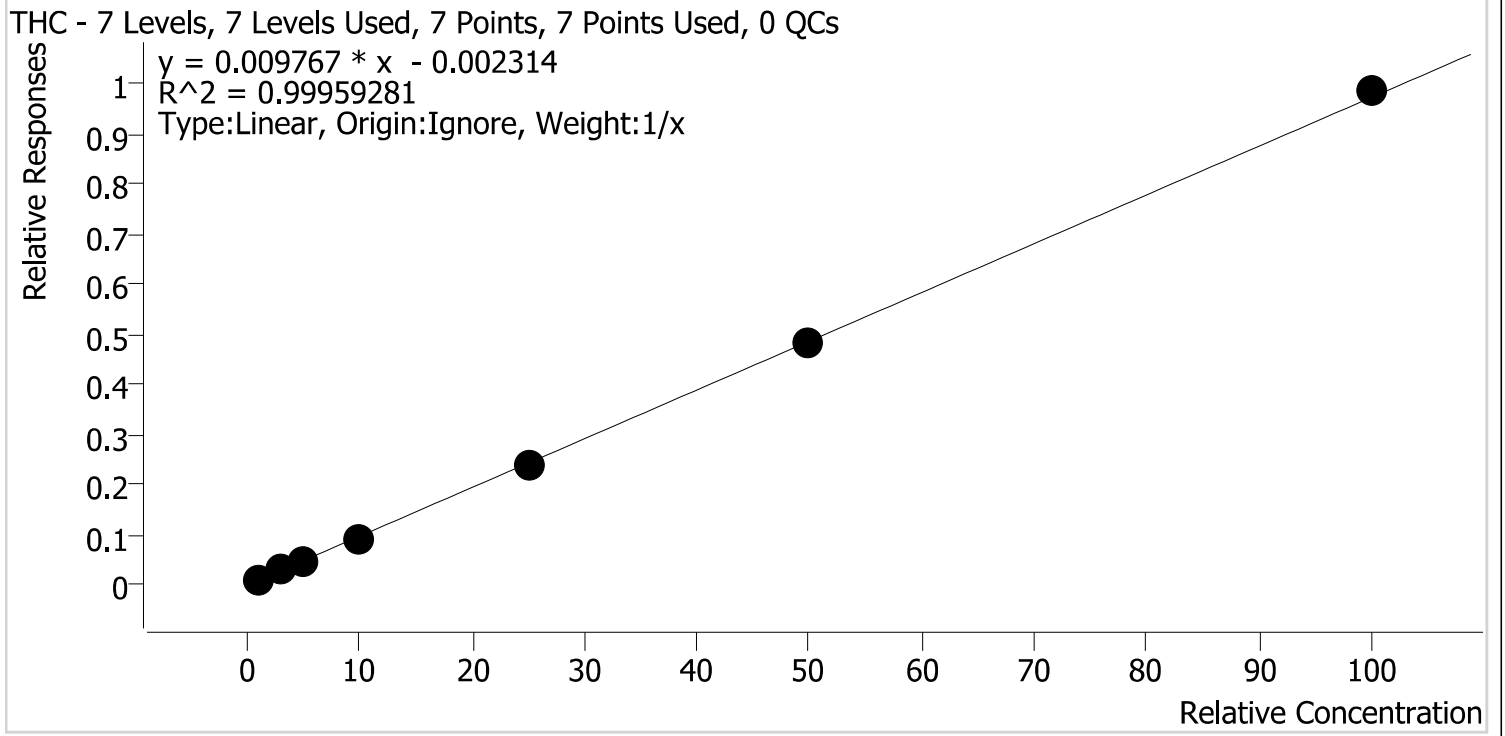
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.468	127131	22.47	7.0 Low	∞	972873	3.6587 ng/ml
THC-COOH	1.474	73356	∞	66.6	∞	205368	13.9126 ng/ml
THC	3.315	306719	1525.28	27.1	∞	6822521	4.8401 ng/ml

AA



AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2021\AM 27-28\090921 AM 27 AG\QuantResults\AM 27.batch.bin
Last Cal. Update 9/10/2021 2:52 PM
Analyst Name ISP\datastor
Analyte THC **Internal Standard** THC-D3



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
MJQ_Cal 1	1	✓	1.0	1.1	111.4
MJQ_Cal 2	2	✓	3.0	2.9	97.8
MJQ_Cal 3	3	✓	5.0	4.8	96.3
MJQ_Cal 4	4	✓	10.0	9.5	95.3
MJQ_Cal 5	5	✓	25.0	24.6	98.5
MJQ_Cal 6	6	✓	50.0	49.7	99.4
MJQ_Cal 7	7	✓	100.0	101.3	101.3

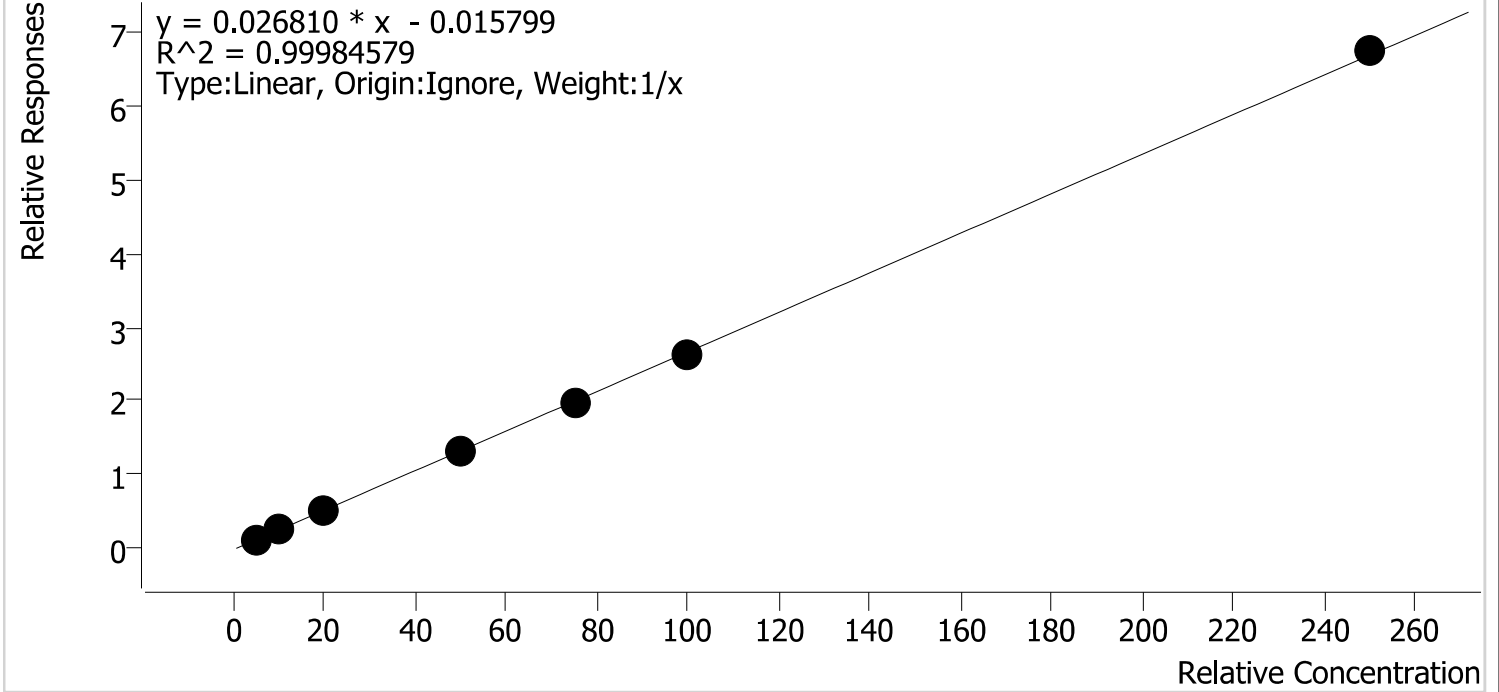
AA



AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2021\AM 27-28\090921 AM 27 AG\QuantResults\AM 27.batch.bin
Last Cal. Update 9/10/2021 2:52 PM
Analyst Name ISP\datastor
Analyte THC-COOH **Internal Standard** THC-COOH-D9

THC-COOH - 7 Levels, 7 Levels Used, 7 Points, 7 Points Used, 0 QCs



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
MJQ_Cal 1	1	✓	5.0	5.1	101.2
MJQ_Cal 2	2	✓	10.0	9.9	99.3
MJQ_Cal 3	3	✓	20.0	20.5	102.3
MJQ_Cal 4	4	✓	50.0	49.2	98.4
MJQ_Cal 5	5	✓	75.0	74.2	99.0
MJQ_Cal 6	6	✓	100.0	99.0	99.0
MJQ_Cal 7	7	✓	250.0	252.1	100.8

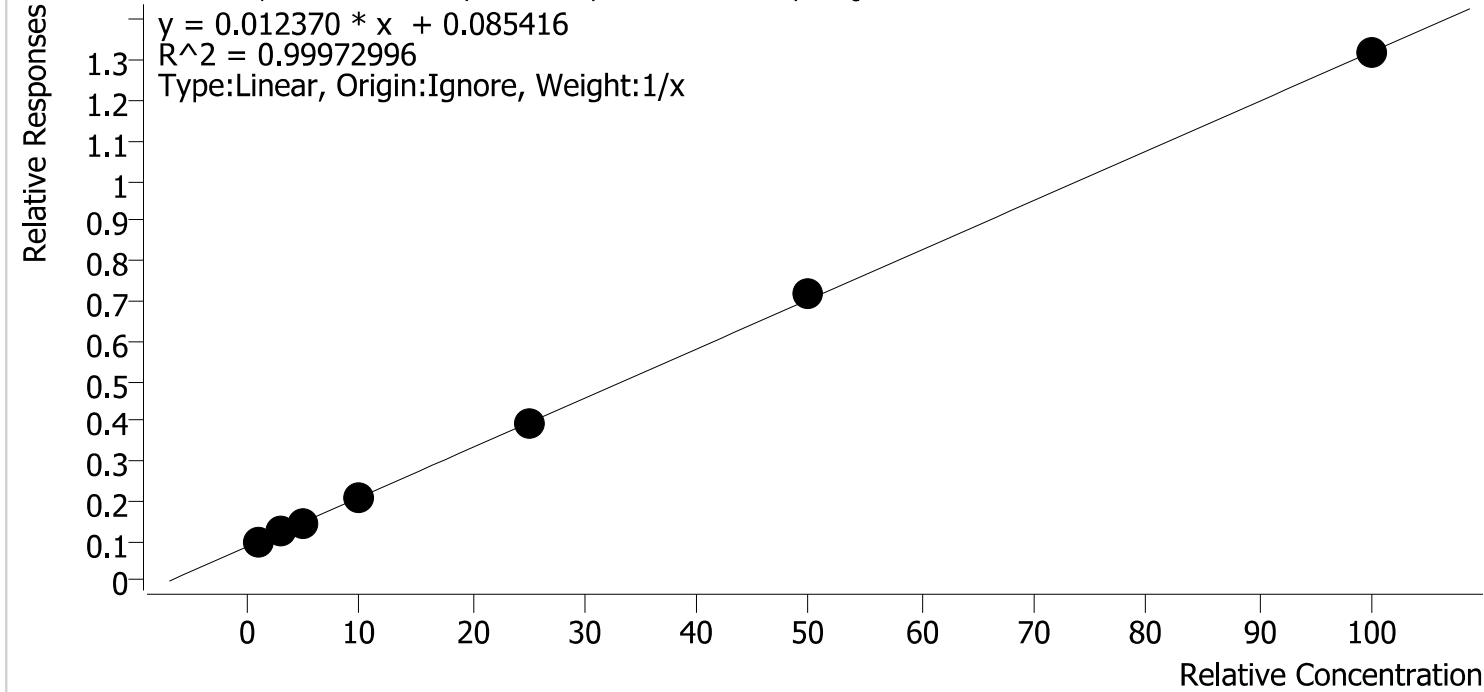
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AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2021\AM 27-28\090921 AM 27 AG\QuantResults\AM 27.batch.bin
Last Cal. Update 9/10/2021 2:52 PM
Analyst Name ISP\datastor
Analyte THC-OH **Internal Standard** THC-OH-D3

THC-OH - 7 Levels, 7 Levels Used, 7 Points, 7 Points Used, 0 QCs



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
MJQ_Cal 1	1	✓	1.0	1.1	105.1
MJQ_Cal 2	2	✓	3.0	3.1	101.9
MJQ_Cal 3	3	✓	5.0	4.8	96.3
MJQ_Cal 4	4	✓	10.0	9.6	95.8
MJQ_Cal 5	5	✓	25.0	24.9	99.5
MJQ_Cal 6	6	✓	50.0	50.9	101.7
MJQ_Cal 7	7	✓	100.0	99.8	99.8

Compound not evaluated due to possible interfering peak

AA

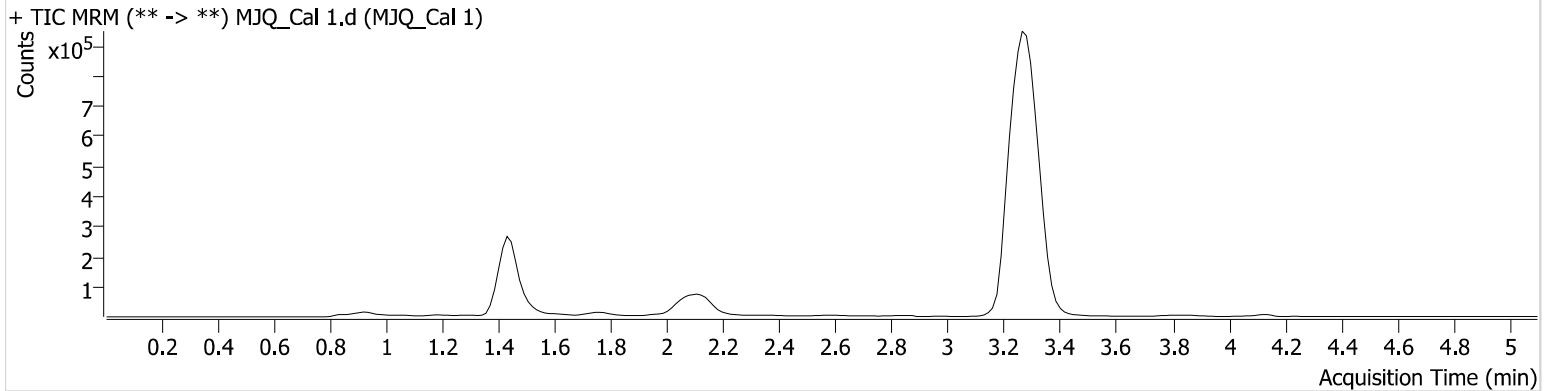


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\090921 AM 27 AG\QuantResults\AM 27.batch.bin
Calibration Last Update 9/10/2021 2:52:11 PM

Instrument	Falco (069901)	Data File	MJQ_Cal 1.d
Type	Cal	Sample	MJQ_Cal 1
Acq. Method	AM 27 THCQ.m	Operator	Amber Gerheart
Sample Position	P1-A1	Comment	
Injection Volume	10		
Acq. Date-Time	9/9/2021 12:33:28 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.498	98184	∞	3.2 Low	12.84	997634	1.0509 ng/ml Low
THC-COOH	1.459	26633	∞	64.7	304.64	222237	5.0593 ng/ml
THC	3.285	58568	248.17	32.3	∞	6834953	1.1143 ng/ml

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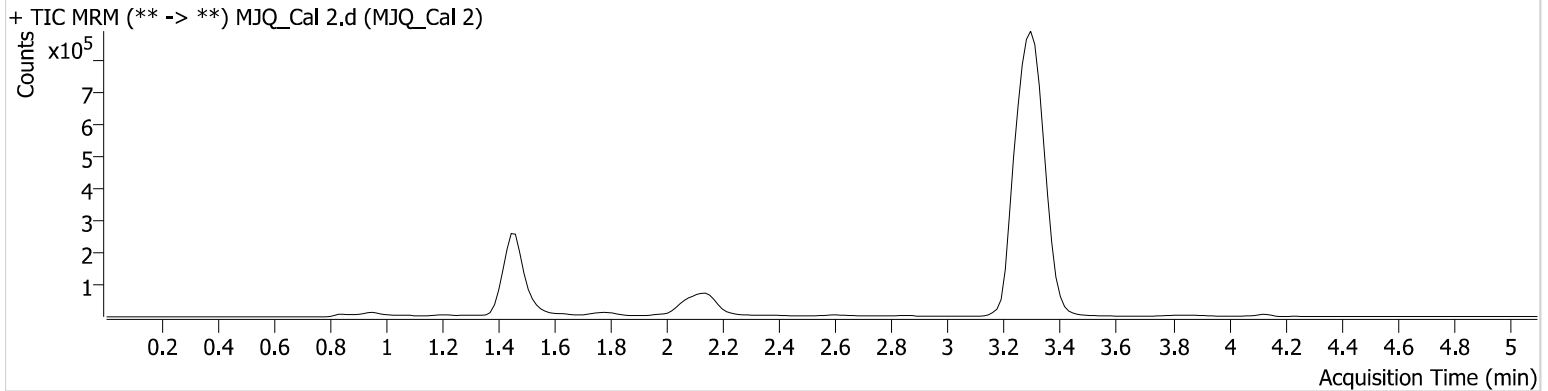


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\090921 AM 27 AG\QuantResults\AM 27.batch.bin
Calibration Last Update 9/10/2021 2:52:11 PM

Instrument	Falco (069901)	Data File	MJQ_Cal 2.d
Type	Cal	Sample	MJQ_Cal 2
Acq. Method	AM 27 THCQ.m	Operator	Amber Gerheart
Sample Position	P1-B1	Comment	
Injection Volume	10		
Acq. Date-Time	9/9/2021 12:41:14 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.513 High	116926	∞	5.5 Low	∞	948914	3.0561 ng/ml
THC-COOH	1.489	52953	∞	64.7	764.96	211488	9.9286 ng/ml
THC	3.315	166764	∞	28.6	191.87	6328670	2.9350 ng/ml

AG

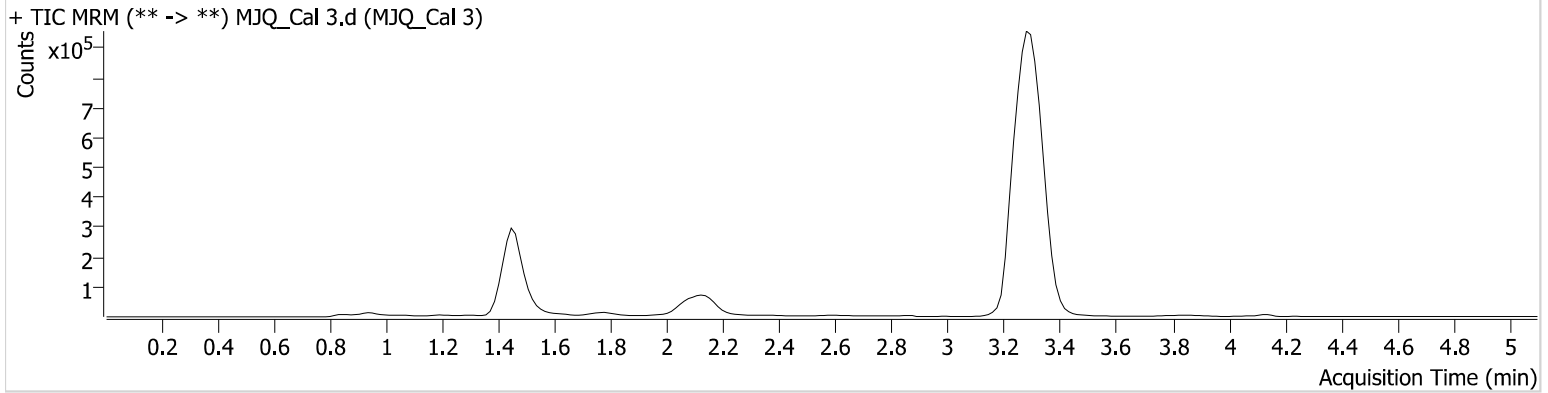


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\090921 AM 27 AG\QuantResults\AM 27.batch.bin
Calibration Last Update 9/10/2021 2:52:11 PM

Instrument	Falco (069901)	Data File	MJQ_Cal 3.d
Type	Cal	Sample	MJQ_Cal 3
Acq. Method	AM 27 THCQ.m	Operator	Amber Gerheart
Sample Position	P1-C1	Comment	
Injection Volume	10		
Acq. Date-Time	9/9/2021 12:48:51 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.468	143369	∞	6.8 Low	∞	989063	4.8130 ng/ml
THC-COOH	1.474	116092	∞	61.7	∞	217948	20.4575 ng/ml
THC	3.315	295224	∞	27.2	∞	6603715	4.8143 ng/ml

AG

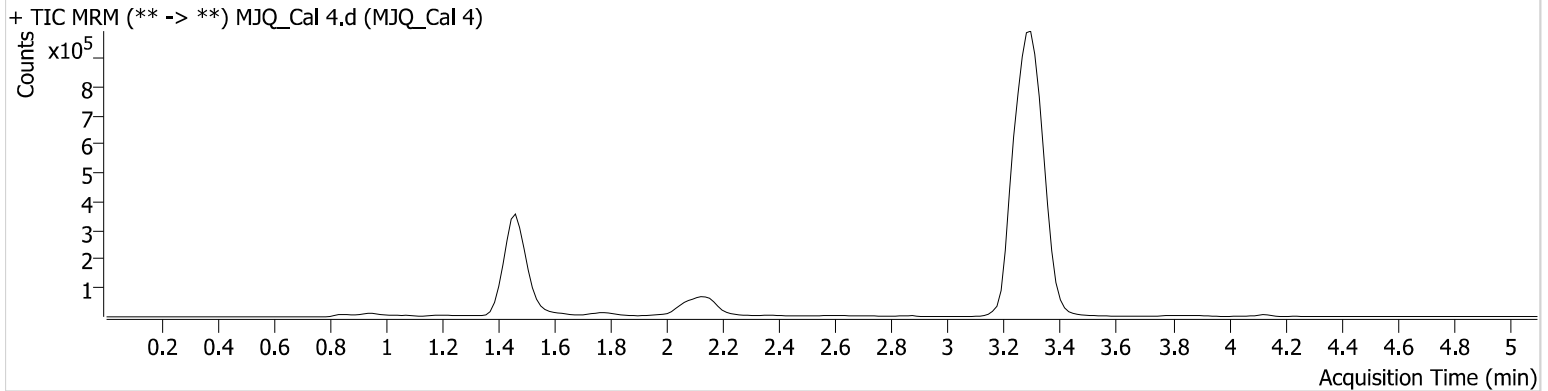


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\090921 AM 27 AG\QuantResults\AM 27.batch.bin
Calibration Last Update 9/10/2021 2:52:11 PM

Instrument	Falco (069901)	Data File	MJQ_Cal 4.d
Type	Cal	Sample	MJQ_Cal 4
Acq. Method	AM 27 THCQ.m	Operator	Amber Gerheart
Sample Position	P1-D1	Comment	
Injection Volume	10		
Acq. Date-Time	9/9/2021 12:56:26 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.468	205328	∞	8.8	49.48	1006901	9.5798 ng/ml
THC-COOH	1.489	288018	∞	64.8	∞	220955	49.2102 ng/ml
THC	3.300	604542	3103.26	26.7	∞	6663956	9.5256 ng/ml

AA

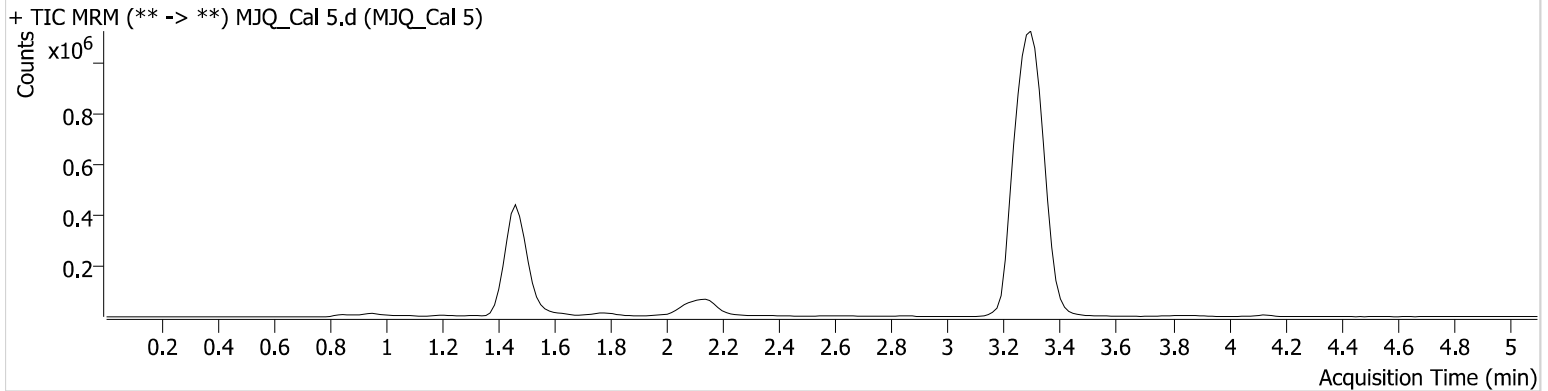


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\090921 AM 27 AG\QuantResults\AM 27.batch.bin
Calibration Last Update 9/10/2021 2:52:11 PM

Instrument	Falco (069901)	Data File	MJQ_Cal 5.d
Type	Cal	Sample	MJQ_Cal 5
Acq. Method	AM 27 THCQ.m	Operator	Amber Gerheart
Sample Position	P1-E1	Comment	
Injection Volume	10		
Acq. Date-Time	9/9/2021 1:04:01 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.468	392847	∞	11.8 High	∞	999417	24.8708 ng/ml
THC-COOH	1.489	420452	∞	64.1	∞	212995	74.2194 ng/ml
THC	3.300	1546589	∞	25.8	∞	6493809	24.6225 ng/ml

AG

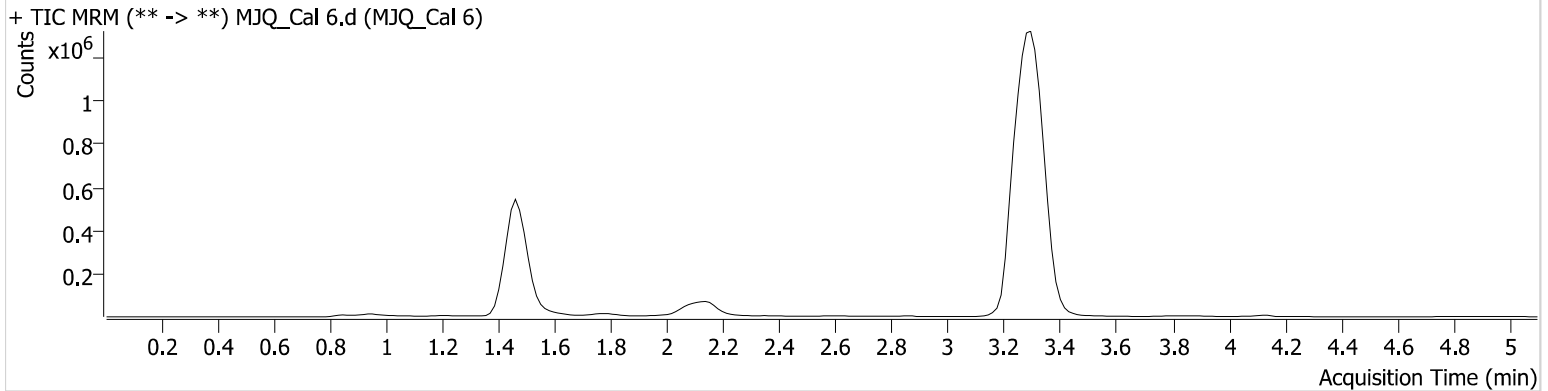


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\090921 AM 27 AG\QuantResults\AM 27.batch.bin
Calibration Last Update 9/10/2021 2:52:11 PM

Instrument	Falco (069901)	Data File	MJQ_Cal 6.d
Type	Cal	Sample	MJQ_Cal 6
Acq. Method	AM 27 THCQ.m	Operator	Amber Gerheart
Sample Position	P1-F1	Comment	
Injection Volume	10		
Acq. Date-Time	9/9/2021 1:11:37 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.468	695936	∞	12.8 High	∞	973776	50.8687 ng/ml
THC-COOH	1.489	545170	∞	68.4	∞	206592	99.0192 ng/ml
THC	3.300	2957826	∞	26.4	∞	6120583	49.7178 ng/ml

AG

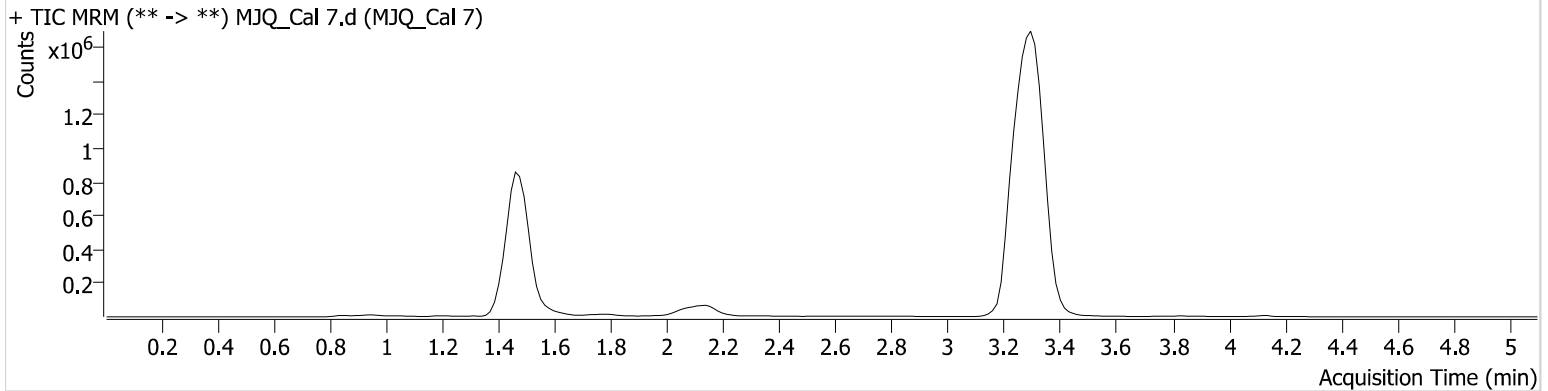


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\090921 AM 27 AG\QuantResults\AM 27.batch.bin
Calibration Last Update 9/10/2021 2:52:11 PM

Instrument	Falco (069901)	Data File	MJQ_Cal 7.d
Type	Cal	Sample	MJQ_Cal 7
Acq. Method	AM 27 THCQ.m	Operator	Amber Gerheart
Sample Position	P1-G1	Comment	
Injection Volume	10		
Acq. Date-Time	9/9/2021 1:19:12 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.453	1219340	∞	13.7 High	∞	924101	99.7607 ng/ml
THC-COOH	1.489	1282014	∞	68.2	∞	190123	252.1059 ng/ml
THC	3.300	5744048	∞	26.5	∞	5821165	101.2705 ng/ml